graphic analysis detected traces of Cr^{+++} in all organs, with most in thyroid and spleen.

The relationship between the activation by Mn^{++} and that by $Mg^{++}+Cr^{+++}+HDP$ is curious. The variability in the ratio of these two activities (see Table 6) at first indicated that two different enzymes might be concerned, but the absence of any marked change in the ratio during the purification of the enzyme, and the variability in some other properties of the enzyme between one preparation and the next, suggest that this is not so. Further work on the interrelations between the activations by various metals and combinations is proceeding.

SUMMARY

1. The greatest activity of phosphoglucomutase is found to occur only in the simultaneous presence of three activators, hexosediphosphate, Mg^{++} and a second metal.

2. The second metal may be Al^{+++}, Cr^{+++}, Pb^{++}, Fe^{+++}, or Ce^{+++}; some other metals also show smaller activity.

3. In this system Mg^{++} cannot be replaced by Mn^{++} or Co^{++}. The maximal activity is some ten times that previously observed with Mn^{++} as the only activator.

4. Of all the 'second metals' studied, only Cr^{+++} shows any activity in the absence of Mg^{++}, and that only at relatively high concentrations.

5. Consideration of affinities suggests that if this 'two-metal' activation has any physiological importance, then Cr^{+++} and Mg^{++} are the metals concerned.

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The Effect of the Peroxide Concentration and other Factors on the Decomposition of Hydrogen Peroxide by Catalase

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When catalase is added to hydrogen peroxide there is an initial rapid evolution of oxygen which lasts for about 2 min. After this oxygen is given off at a steady rate which slowly decreases in the course of about an hour. This is not necessarily due to a decrease in the peroxide concentration, since it is quite marked in experiments where there is a large excess of peroxide (Morgulis, Beber & Rabkin, 1926; George, 1947). The first problem in studying the kinetics of the reaction is to determine to what extent destruction of the enzyme is responsible for these changes in the rate as the reaction proceeds.

The results of some of the early investigations are difficult to interpret because only the total amount of oxygen evolved from a given amount of catalase and peroxide is recorded, so that the initial rapid reaction and the steady evolution cannot be distinguished. Provided the catalase is not present in excess the initial rapid reaction represents a small proportion of the total reaction possible, and as this condition obtained in most of the early investigations the results refer mainly to the subsequent steady rate. This is found to be directly proportional to the enzyme concentration, whereas the variation with peroxide concentration is more complicated. Above an optimum concentration as the peroxide is increased the reaction proceeds more slowly (Evans, 1907; Morgulis *et al.* 1926).

There is no doubt that the gradual decrease in the rate, after the initial rapid reaction is over, is due to enzyme destruction, and several kinetic equations have been developed to account for it in the papers of Yamasaki (1920), Morgulis (1921), Northrop (1924–5) and Williams (1927–8). There

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remain two problems. Enzyme destruction may be one factor determining the variation of the steady rate with peroxide concentration. Partial enzyme destruction may be responsible for the transition from the initial rapid rate to the slower steady rate—the transition from α- to β-activity which was examined in a previous paper (George, 1947).

Experiments have been carried out to establish the variation of the reaction rate with enzyme and peroxide concentration by measuring the rates in the early stages of the reaction for different initial concentrations. With this method it is possible to determine the true kinetics of the H_2O_2 decomposition unaffected by the slow destruction of the enzyme, which makes the kinetic analysis of H_2O_2 concentration changes during a prolonged experiment extremely difficult. In the second part of the paper experiments are described which show that enzyme destruction is not responsible for either the kinetics of the steady reaction or the transition from α- to β-activity.

EXPERIMENTAL

Materials and methods

Enzyme preparations. A purified preparation of liver catalase (CL 1) was obtained from horse liver by the method described by Keilin & Hartree (1945). Erythrocyte catalase was prepared from 2 l. fresh defibrinated horse blood, the red corpuscles being washed and plasmolyzed, and the haemoglobin removed by the Tsuchihashi method as described by Keilin & Mann (1940) in their preparation of carbonic anhydrase. Catalase was extracted from the resulting clear yellowish fluid by the process used for liver catalase, i.e. adsorption on calcium triphosphate gel followed by elution and fractional precipitation with $(NH_4)_2SO_4$; this was done twice, NH_4OH being used for the first elution and Na_2HPO_4 for the second. Two specimens of erythrocyte catalase, CE 1 and CE 2 were obtained; CE 1 was the purer sample, CE 2 being extracted from the residues of the second adsorption. These specimens of catalase were analyzed for Fe, determined colorimetrically with 2:2′-dipyridyl, and for haemin, determined spectroscopically as pyridine haemochromogen (Keilin & Hartree, 1936), and compare favourably as regards purity with those used by Keilin & Hartree (1936) (Table 1). The low values for haemin are probably due to partial removal of the prosthetic group from the enzyme in the final stages of the purification. Two other specimens of liver catalase, CL 2 and CL 3, kindly provided by Dr E. F. Hartree, have also been used. Dilute catalase solutions were made each day from the sterile stock solutions (see Table 1), and stored in ice and water in a thermos flask. Some dilutions were made in 0·02% gelatin which had no effect on the rate of the reaction, but reduced inactivation of the enzyme during storage.

Table 1. *Analysis of catalase specimens*

(CL 1 liver catalase, CE 1 and CE 2 erythrocyte catalase.)

Analyses	Specimens CL 1	CE 1	CE 2
Dry weight (mg./ml.)	34·1	5·2	5·4
Haemin (% on dry wt.)	0·51	0·47	0·50
Total iron (% on dry wt.)	0·14	0·11	0·09
Haemin iron (as % of total iron)	31	41	48

Keilin & Hartree (1936) found for three specimens 0·52, 0·55 and 0·37% haemin by weight.

A plasmolyzed red cell preparation used in later experiments was made by taking 1·0 ml. of a red cell suspension from defibrinated horse blood, which had been washed twice with 0·9% NaCl and spun down, and diluting to 250 ml. with distilled water.

Procedure. The O_2 evolution was measured as previously described in Barcroft manometers at 20° and in the pressure-gauge apparatus at 0° and 19·3–19·5° using the 'boat technique' of Meldrum & Roughton (1934) and George (1947). For the experiments in which dilute buffer solution was added to the reaction mixture during a run, flasks with a side tube of 1·5 ml. capacity were used. In the experiments where H_2O_2 was added during a run, two dangling tubes were used; the first with a short platinum hook contained dilute catalase solution and the second with a long platinum hook contained the required amount of '100 vol.' H_2O_2. Control experiments with no catalase present showed the blank reaction to be negligible in the concentration range 0–1·0 M-H_2O_2 where '20 vol.' H_2O_2 was used. For the range 1·0–5·0 M-H_2O_2, '100 vol.' H_2O_2 was used, the contribution of the blank being determined at each concentration, and subtracted from the experimental value. Before use the '100 vol.' H_2O_2 was brought to about pH 6·0 by adding a few drops of NaOH, bromocresol purple being used as indicator. In the majority of the experiments A.R. H_2O_2 was employed, but in one series a freshly prepared sample was used.

In all the experiments the total volume of O_2 evolved was very small compared with the H_2O_2 concentration which can be assumed to remain constant throughout each experiment. Protocols for the two methods of measuring gas evolution in experiments in which the peroxide concentration was varied are given below. *Barcroft manometers*: left-hand flask, 3·30 ml. H_2O; right-hand flask, 0·30 ml. 0·125 M-phosphate buffer, pH 5·85; 0·10 ml. 2% gelatin solution; 0·10 ml. dilute catalase solution in dangling tube; H_2O and H_2O_2 to 3·30 ml. to give required molarity. *Boats*: Side '*a*': 1·0 ml. H_2O; 0·5 ml. dilute catalase solution; 0·5 ml. 0·2 M-buffer solution; side '*b*': 2·0 ml. H_2O and H_2O_2 to give required molarity.

Buffers. To determine the effect of different buffer solutions of the same pH, Sorensen's citrate buffer and Clark & Lubs's phthalate buffer were used as well as Sorensen's phosphate buffer. The solutions were prepared as described by Clark (1925); the pH values, determined electrometrically, were 5·86, 5·84 and 5·85 at 20° respectively, the apparatus having been standardized with 0·05 M-potassium hydrogen phthalate solution, pH = 3·97 at 20°.

RESULTS

Variation of the oxygen evolution rate with enzyme concentrations

Barcroft manometers and the catalase specimen CL 2 were used at 20°, the peroxide concentration being kept at a constant value and the enzyme con-

centration varied. For 0·01, 0·03 and 0·5 M-H_2O_2 a dilute catalase solution was employed and between 0·02 and 0·30 ml. placed in the dangling tube; for 4·8 M-H_2O_2 a solution 10 times as concentrated was used. The results are given in Table 2, and show that for each peroxide concentration the rate is directly proportional to the catalase concentration.

Table 2. *Variation of the oxygen-evolution rate with enzyme concentration*

(Barcroft manometers at 20°; catalase CL2 and 0·3 ml. 0·125M-phosphate buffer (pH 5·85) present in each experiment. 10 Enzyme units (e.u.) equivalent to $1·84 \times 10^{-4}$ mg. CL2.)

H_2O_2 (M)	E.u.	Rate at times after start (μl. O_2/min.)	Rate/e.u.
		0–1 min.	
0·01	5	20	4·0
	10	42	4·2
	15	58	3·9
	20	73	3·7
	25	90	3·6
	30	111	3·7
		2–3 min.	
0·03	2	14	7·0
	4	31	7·7
	6	43	7·2
	8	56	7·0
	10	68	6·8
		2–3 min.	
0·5	5	21	4·3
	10	42	4·2
	15	64	4·3
	20	32	4·1
	25	109	4·4
		2–3 min.	
4·8	10	7·5	0·75
	30	19·0	0·63
	50	29	0·58
	70	42	0·60
	90	58	0·64

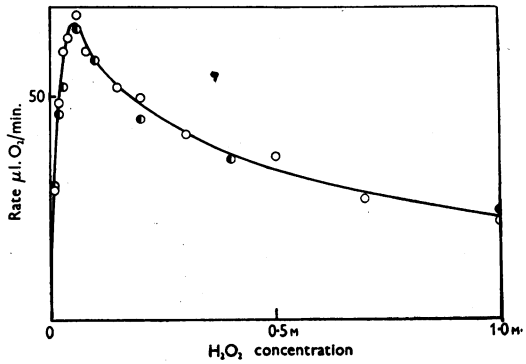

Fig. 1. Variation of the O_2-evolution rate, between the second and third minute from the start of the reaction, with the peroxide concentration. Measured with Barcroft differential manometers at 20° in the presence of 0·3 ml. 0·125M-phosphate buffer, pH 5·85; ○—○, with liver catalase specimen CL1, $1·84 \times 10^{-4}$ mg.; ◐—◐, with erythrocyte catalase specimen CE1, $1·66 \times 10^{-4}$ mg.

Variation of the oxygen-evolution rate by liver or erythrocyte catalase with peroxide concentration

Barcroft manometers were used at 20°, the enzyme concentration being kept constant and the rate of O_2 evolution with different initial concentrations of peroxide determined. The O_2 evolved was measured for 10 min. at 0·5 and 1 min. intervals, and from the figures obtained the rate over any given interval could be calculated. Fig. 1 shows the rates for liver

Table 3. *Variation of the oxygen-evolution rate with peroxide concentration*

(Barcroft differential manometers at 20°; $1·84 \times 10^{-4}$ mg. catalase CL2 and 0·3 ml. 0·125M-phosphate buffer (pH 5·85) present in each experiment.)

H_2O_2 (M)	Rate at different times after beginning of reaction (μl. O_2/min.)				
	0–0·5 min.	0·5–1 min.	1–2 min.	2–3 min.	4–5 min.
0·01	38	44	38	30	21
0·02	62	96	68	51	37
0·03	108	134	81	68	48
0·04	124	144	94	71	50
0·05	174	166	108	80	61
0·06	186	178	113	83*	63
0·07	214	222	119*	83*	65*
0·08	244	262*	119*	82	64
0·10	234	188	98	73	61
0·15	210	166	84	68	58
0·20	252	130	70	59	51
0·30	264	102	59	52	47
0·50	274*	71	47	42	37
0·70	226	56	35	33	30
1·00	180	62	34	29	27
2·00	100	25	15·5	13·5	13·5
3·40	66	21	9·5	8·5	7·5
4·80	54	14	7·5	7·5	7·5

* Indicates maximum rate.

catalase CL 1 and erythrocyte catalase CL 1 over the range 0–1 M-H_2O_2, between the second and third minute from the start of the reaction, at which time most of the initial 'burst' is over (George, 1947). There is a very marked maximum in the rate at about 0·06 M-H_2O_2, and the catalase specimens from the two different sources give identical curves. The inhibition of the reaction at high H_2O_2 concentrations is very marked, for with 1·0, 2·0 and 4·0 M-H_2O_2 the rate is 32, 21 and 8 % respectively of the maximum rate at 0·06 M-H_2O_2.

No attempt has been made to compare the activities of the enzyme preparations on the basis of their haemin content. Catalase is an extremely active enzyme and the presence of about 1×10^{-6} g. of inactive protein, and 1×10^{-8} g. haemin or haemin degradation products is very unlikely to affect the rate of the reaction. For the kinetic analysis it is sufficient to show that with identical rates for the two specimens at, for instance, 1·0 M-H_2O_2, the same variation with H_2O_2 concentration is shown by each.

Similar results with liver catalase CL 2 at 20° are shown in Table 3 which shows the initial rapid evolution of O_2 followed by a lower steady rate which was discussed in a previous paper (George, 1947). The maximum initial rate, as given by the O_2 evolved during the first half minute of the reaction, occurs at a high peroxide concentration between 0·3 and 0·7 M-H_2O_2, whereas the maximum steady rate, measured by the O_2 evolved between the fourth and fifth minute from the start of the reaction, occurs at a much lower peroxide concentration—about 0·06–0·08 M-H_2O_2. The initial rates will be examined more fully in a later paper.

Variation of the oxygen-evolution rate with peroxide or enzyme concentration using plasmolyzed cells

To investigate the effect of impurities on the kinetics of the reaction, comparative experiments were carried out in the pressure gauge apparatus at 0° with erythrocyte catalase CE 2 ($1{\cdot}73 \times 10^{-3}$ mg.) and a suspension of plasmolyzed red blood cells (1/250 dilution, 0·2 ml.).

Fig. 2 shows that the same type of curve is given by the crude and the purified enzyme, and that the maximum occurs at the same H_2O_2 concentration. Table 4 shows that the rate is directly proportional to the concentration of lyzed cells. Since identical results are obtained with the purified enzyme extract and plasmolyzed red blood cells, the kinetics of the enzyme reaction are not affected by chance impurities in the enzyme preparation.

Effect of different samples of hydrogen peroxide on the oxygen-evolution rate

The inhibition of the reaction observed at high peroxide concentrations might be due to an inhibitory substance present in the specimen of H_2O_2

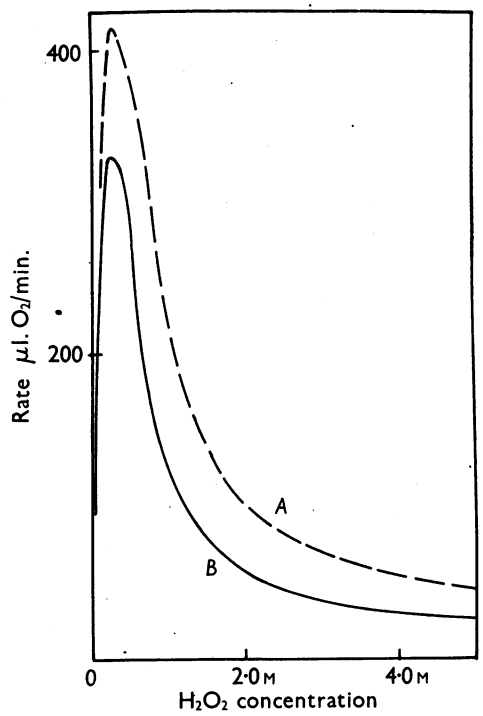

Fig. 2. A comparison of the variation of the O_2-evolution rate with peroxide concentration for a dilute suspension of lyzed red blood cells (curve *A*) and the purified erythrocyte catalase specimen CE 2 (curve *B*). Measurements of the rate in μl. O_2/min. made between 10 and 40 sec. from the start of the reaction with the pressure-gauge apparatus at 0°; 0·5 ml. 0·2 M-phosphate buffer (pH 5·85); $1{\cdot}73 \times 10^{-3}$ mg. catalase (CE 2) or 0·2 ml. 1/250 dilution lyzed cells present.

Table 4. *Variation of the oxygen-evolution rate with the concentration of plasmolyzed red blood cells*

(Pressure gauge apparatus at 0° in the presence of 0·5 ml. 0·2 M-phosphate buffer, pH 5·85. Lyzed cell concentration (L.C.) in ml. 1/250 dilution. Rate measured between 10 and 40 sec. from the start of the reaction.)

H_2O_2 (M)	L.C. (ml.)	Rate (μl. O_2/min.)	Rate/ml. enzyme
0·2	0·05	90	1800
	0·10	180	1800
	0·15	295	1980
	0·20	400	2000
1·0	0·1	85	850
	0·2	200	1000
	0·3	305	1020
	0·4	405	1010
5·0	0·2	40	200
	0·4	120	300
	0·6	180	300
	0·8	210	262

used. A sample of H_2O_2 was, therefore, prepared in the laboratory from Na_2O_2 and H_2SO_4 and purified by vacuum distillation (Kilpatrick, Reiff & Rice, 1926). Neither the standard sample nor the fresh sample showed any fluorescence when illuminated with strong ultraviolet light. Liver catalase CL2 was used for the determinations which were carried out in Barcroft manometers at 20°. Table 5 shows

Table 5. *Comparison of the oxygen evolved from the standard peroxide and freshly prepared peroxide*

(Barcroft manometers at 20°; $1{\cdot}84 \times 10^{-4}$ mg. catalase CL2 and 0·3 ml. 0·125 M-phosphate buffer (pH 5·85) present in each experiment. The average of five separate determinations given for each sample.)

H_2O_2 (M)	Time after start (min.)	O_2 evolved (μl./min.) Standard H_2O_2	Fresh H_2O_2
0·5	1–2	30·8	31·5
	2–3	29·8	29·6
	4–5	24·5	24·0
0·07	1–2	73·7	73·0
	2–3	52·5	51·4
	4–5	40·3	39·6

that there is a precise correspondence between the results for both specimens of H_2O_2, so the inhibition of the reaction at high H_2O_2 concentrations is not due to any inhibitory substance present in the peroxide.

Effect of different buffer solutions of the same pH *on the oxygen-evolution rate by catalase*

Anions are known to inhibit the decomposition of H_2O_2 by catalase owing to competition for the ferric iron of the haemin between OH^- and the other anions (Agner & Theorell, 1946), which is more marked the greater the H^+-ion concentration. It was desirable to find out whether the inhibition observed at high H_2O_2 concentrations arises from a complex reaction involving the constituents of the buffer solution. Experiments were made using the pressure-gauge apparatus at 19·3–19·5° with liver catalase CL3 to compare the effect of phosphate, citrate and phthalate buffers of pH values 5·85, 5·86 and 5·84 respectively, at concentrations between 0·005 and 0·1 M. The results are given in Table 6 for two concentrations of substrate (0·1 and 1·0 M-H_2O_2). The results show clearly that at pH 5·84–5·86 the rates are the same at 1·0 M-H_2O_2 and independent of the nature and concentration of the buffer solution used. At 0·1 M-H_2O_2 there may be a slight inhibition with the phthalate buffer, but the variation in the figures is of the same order as the experimental error. With all these buffers the steady rate with 0·1 M-H_2O_2 is between 2·0 and 2·4 times as great as the steady rate with 1·0 M-H_2O_2 as observed in the previous experiments, and there is no systematic fall in the rate as the buffer concentration is increased. This shows clearly that reactions of the anion or cation of the buffer solution do not play any part in determining the kinetics of the peroxide decomposition, and are not responsible for the inhibition of the decomposition observed at high H_2O_2 concentrations.

Reversibility of the inhibition of catalase by high peroxide concentrations

The question whether destruction of enzyme accounts for the reaction kinetics can be settled by experiments in which water or peroxide is added

Table 6. *Comparison of the oxygen-evolution rates with different buffers of the same* pH

(Pressure gauge apparatus at 19·3–19·5°; catalase CL3, $7{\cdot}3 \times 10^{-4}$ mg.)

Buffer Type	pH	Vol. (0·2 M-solution)	Rates (μl. O_2/min.) at time after start of reaction: 0·1 M-H_2O_2 10–60 sec.	0·1 M-H_2O_2 2–4 min.	1·0 M-H_2O_2 10–60 sec.	1·0 M-H_2O_2 2–4 min.
Phosphate	5·85	0·1	950	248	199	109
		0·5	965	240	199	106
		1·0	995	248	208	113
		1·5	990	252	211	117
		2·0	1040	235	—	—
		Av.	**988**	**245**	**204**	**111**
Citrate	5·86	0·1	1060	232	204	113
		0·5	988	226	198	109
		1·0	—	—	—	—
		1·5	—	—	228	114
		2·0	960	218	—	—
		Av.	**996**	**225**	**210**	**112**
Phthalate	5·84	0·1	955	223	202	102
		0·5	955	218	199	100
		2·0	950	215	230	103
		Av.	**953**	**219**	**210**	**102**

during the reaction, for if complete reversibility is observed when the substrate concentration is brought to the optimum, then it is certain that enzyme destruction plays no part in determining the kinetics.

In the dilution experiments a buffer solution was added rather than water alone so that the ionic strength did not change, as an added safeguard that the observed effect is due only to the reaction between catalase and H_2O_2. The experiments were made in Barcroft manometers at 20°, a total of 1·5 ml. being used in each flask: the O_2 evolution was followed for 4·5 or 5 min., each experiment being done in triplicate. Catalase CL3 was employed, 0·1 ml. ($1·9 \times 10^{-5}$ mg.) being placed in the dangling tube: the concentration after mixing was about one fifth of that used in the previous experiments. With the usual amount of catalase the amount of O_2 evolved during the 5 min. of this experiment might amount to one quarter of the total H_2O_2 present, giving a substrate concentration below the optimum which would tend to mask the increase in the rate which is being looked for. Measurements of O_2 evolution were carried out at 0·01, 0·06, 0·36 and 1·46 M-H_2O_2, the appropriate blanks for the H_2O_2 alone being subtracted from the observed values. Two sets of experiments were made in which dilute buffer solution was added during the reaction, the flask with a side arm of capacity about 1·5 ml. being used. Typical protocols are given.

(*a*) *Concentration change from* 0·6 *to* 0·06 M-H_2O_2. Left-hand flask: 1·5 ml. H_2O. Right-hand flask: 0·094 ml. 1·92 N-H_2O_2, 0·06 ml. 0·2 M-phosphate buffer (pH 5·85), 0·10 ml. dilute catalase solution in dangling tube; total = 0·25 ml. 0·36 M-H_2O_2; side arm, 0·95 ml. H_2O, 0·30 ml. 0·2 M-phosphate buffer (pH 5·85); total 1·25 ml.; after mixing total volume = 1·50 ml. 0·06 M-H_2O_2. With this arrangement the evolution of O_2 can first be determined for 0·25 ml. of 0·36 M-H_2O_2, and then, by allowing the dilute buffer solution to run into the flask by rotating the side arm, measurements can be continued for 1·5 ml. of 0·06 M-H_2O_2; any alteration in the rate is to be attributed only to the change in H_2O_2 concentration. This was checked by an experiment where the H_2O_2 concentration was kept constant (Fig. 3, curve *A*), the O_2 evolution from 0·25 ml. of 0·36 M-H_2O_2 with 0·10 ml. dilute catalase being the same as that from 1·50 ml. of 0·36 M-H_2O_2 with 0·10 ml. dilute catalase, i.e. the same amount but one sixth the previous concentration. Curve *B* gives the O_2 evolution for 1·50 ml. of 0·06 M-H_2O_2 with 0·10 ml. dilute catalase. In experiments illustrated by curves *C* and *D* the reaction was started with 0·25 ml. of 0·36 M-H_2O_2 and after 2 and 1 min. respectively 1·25 ml. of dilute buffer solution were added according to the protocol above; in both cases the O_2 evolution after dilution increases and follows the points on curve *B*, the appropriate curve for the lower H_2O_2 concentration. The inhibition observed with 0·36 M-H_2O_2 can, therefore, be reversed by dilution during the reaction, the reversibility being quantitative to within experimental error. The rates of O_2 evolution, μl. O_2/min., measured over the period 2–4·5 min. from curves *A*, *B* and *C* are 7·0, 10·5 and 10·4 respectively. Curve *D*,

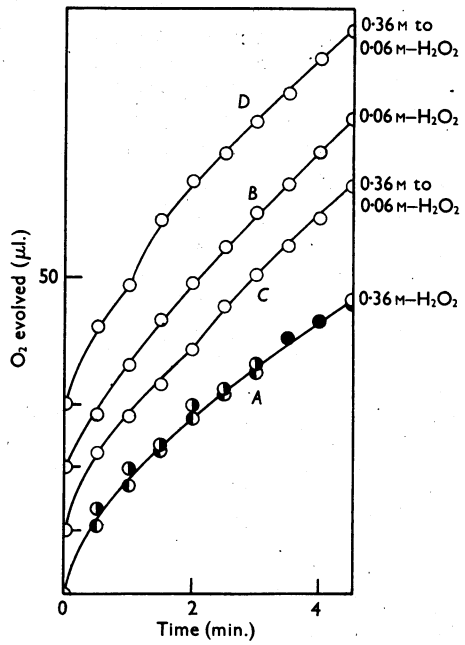

Fig. 3. O_2-evolution curves for 0·36 and 0·06 M-H_2O_2 with $1·9 \times 10^{-5}$ mg. of catalase (CL3) in each experiment. Curve *A*: 0·36 M-H_2O_2, 0·25 ml. solution points ◑; 1·50 ml. solution points ◐. Curve *B*: 0·06 M-H_2O_2, 1·50 ml. solution. Curve *C*: from 0 to 2 min. 0·25 ml. 0·36 M-H_2O_2, then 1·25 ml. dilute buffer solution added from a side arm giving 1·50 ml. 0·06 M-H_2O_2. Curve *D*: as for curve *C* but addition of dilute buffer solution at the end of the first minute. Measurements made with Barcroft differential manometers at 20° in phosphate buffer (pH 5·85), concentration in each experiment 0·024 M.

illustrating the effect of dilution at the end of the first minute of the reaction, will be discussed later when the possibility of enzyme destruction as the cause of the decay of the initial rapid reaction is examined.

(*b*) *Concentration change from* 0·06 *to* 0·01 M-H_2O_2. The results in Table 3 show too that dilution from 0·06 to 0·01 M-H_2O_2 should cause a marked decrease in the rate. This was tested in a similar way to the foregoing experiments, using 0·32 N-H_2O_2 in place of 1·92 N-H_2O_2. The average results for these separate

determinations of O_2 evolution with each H_2O_2 concentration are given in Fig. 4. Curves *A* and *B* show the O_2 evolution for 1·50 ml. of 0·01 M-H_2O_2 and 0·25 ml. of 0·06 M-H_2O_2, the rate being greater at the higher H_2O_2 concentration; curve *C* gives the O_2 evolution for the reaction starting with 0·25 ml. of 0·06 M-H_2O_2 and adding 1·25 ml. of buffer solution after 2 min., and shows the expected decrease in the rate. The rates of O_2 evolution, measured over the period 2–4·5 min. from curves *B*, *A* and *C* are 10·4, 4·8 and 4·4 μl. O_2/min. respectively.

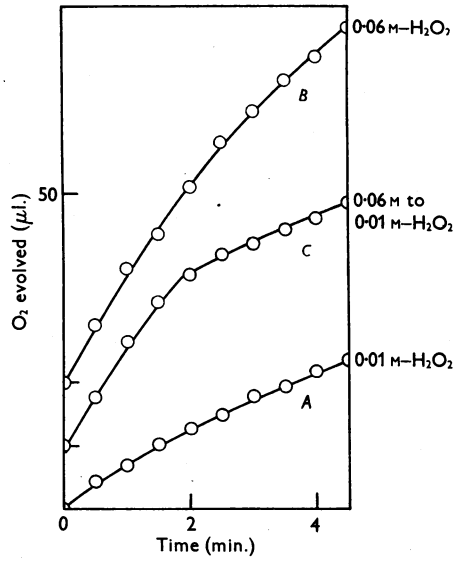

Fig. 4. O_2-evolution curves for 0·01 and 0·06 M-H_2O_2 with $1·9 \times 10^{-5}$ mg. catalase (CL3) in each experiment. Curve *A*: 0·01 M-H_2O_2, 1·50 ml. solution. Curve *B*: 0·06 M-H_2O_2, 0·25 ml. solution. Curve *C*: from 0 to 2 min. 0·25 ml. 0·06 M-H_2O_2, then 1·25 ml. dilute buffer solution added from a side arm giving 1·50 ml. 0·01 M-H_2O_2. Measurements made with Barcroft differential manometers at 20° in phosphate buffer (pH 5·85), concentration in each experiment 0·024 M.

(*c*) *Concentration change from* 0·01 *to* 0·36 M-H_2O_2. Two sets of experiments were then made in which a small amount of '100 vol.' H_2O_2 was added during the reaction from a second dangling tube with a long platinum hook. The dilute catalase solution was contained in the first dangling tube, which had a short platinum hook, and by careful tapping could be dislodged without upsetting the second tube holding the concentrated H_2O_2. *Protocol:* left-hand flask, 1·5 ml. H_2O; right-hand flask, 0·094 ml. 0·32 N-H_2O_2, 0·40 ml. 0·2 M-phosphate buffer (pH 5·85), 0·90 ml. H_2O, 0·10 ml. catalase (= 1·50 ml. 0·01 M-H_2O_2); 0·05 ml. 10·5 M-H_2O_2 in second dangling tube. Total volume = 1·50 ml. 0·36 M-H_2O_2. It is important to show that by increasing the H_2O_2 concentration the rate can also be increased, for this would confirm that the inhibition noted at high H_2O_2 concentrations is not caused by an irreversible oxidative degradation of the enzyme. This can be tested in the concentration range 0·01–0·36 M-H_2O_2: the direct determination of the rates at these two concentrations recorded in Table 3 shows about a twofold increase. Fig. 5 shows the average of three separate determinations of O_2 evolution at each of these H_2O_2 concentrations, curve *A*, 1·50 ml. of 0·01 M-H_2O_2, and curve *B*, 1·50 ml. of 0·36 M-H_2O_2. Curve *C* gives the O_2 evolution for the reaction

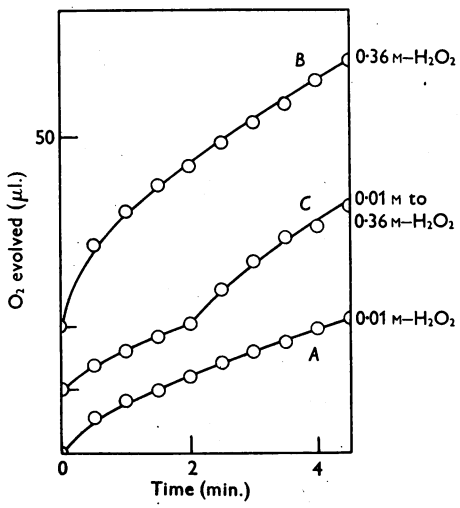

Fig. 5. O_2-evolution curves for 0·01 and 0·36 M-H_2O_2 with $1·9 \times 10^{-5}$ mg. catalase (CL3) in each experiment. Curve *A*: 0·01 M-H_2O_2, 1·50 ml. solution. Curve *B*: 0·36 M-H_2O_2, 1·50 ml. solution. Curve *C*: from 0 to 2 min. 1·50 ml. 0·01 M-H_2O_2, then 0·05 ml. 10·5 M-H_2O_2 added from a second dangling tube giving 1·55 ml. 0·36 M-H_2O_2. Measurements made with Barcroft differential manometers at 20° in phosphate buffer (pH 5·85), concentration in each experiment 0·024 M.

starting with 1·50 ml. of 0·01 M-H_2O_2 and adding 0·05 ml. of 10·5 M-H_2O_2 from the second dangling tube after 2 min. to give 0·36 M-H_2O_2. A marked increase in the rate is observed, which corresponds to that expected for the increase in H_2O_2 concentration. The rates of O_2 evolution measured over the period 2–4·5 min., from curves *A*, *B* and *C*, are 3·7, 7·0 and 7·4 μl. O_2/min. respectively.

(*d*) *Concentration change from* 0·06 *to* 1·46 M-H_2O_2. The inhibitory effect of strong H_2O_2 which was observed in the direct determinations of the rate at different H_2O_2 concentrations (Fig. 2 and Table 3) was confirmed by adding peroxide during the reaction to bring the concentration from 0·06 to 1·46 M-H_2O_2 when the rate should fall to about one third of its previous value. In Fig. 6, curves *A* and *B* refer to the O_2 evolution from 1·50 ml. of 1·46 and

0·06 M-H_2O_2 respectively, while curve *C* gives the O_2 evolved from 1·30 ml. of 0·06 M-H_2O_2 for 2 min., when 0·20 ml. of 10·5 M-H_2O_2 was added and readings taken for a further 3 min. The rate decreases quantitatively corresponding to the peroxide or the nature and concentration of the buffer solution used. The quantitative reversal of the inhibition shown by the addition of dilute buffer solution or peroxide during the reaction shows that the phenomenon is not due to destruction of the enzyme. The remaining problem to be settled is whether or not partial enzyme destruction is responsible for the transition from the initial rapid rate to the steady rate. The experiment recorded in Fig. 3, curve *C*, shows that it is not.

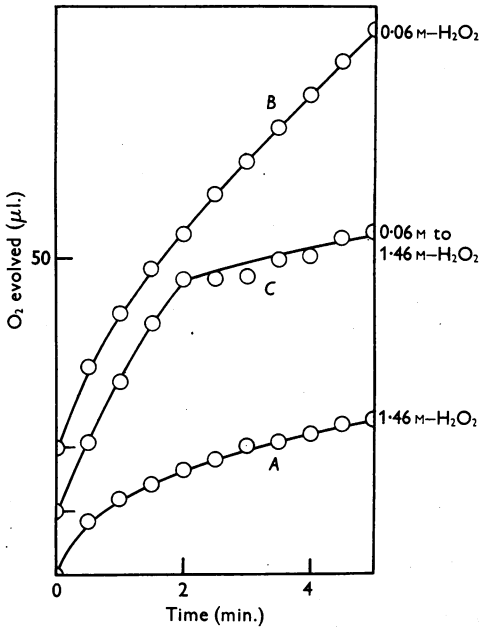

Fig. 6. O_2-evolution curves for 1·46 and 0·06 M-H_2O_2 with $1·9 \times 10^{-5}$ mg. catalase (CL3) in each experiment. Curve *A*: 1·46 M-H_2O_2, 1·50 ml. solution. Curve *B*: 0·06 M-H_2O_2, 1·50 ml. solution. Curve *C*: from 0 to 2 min. 1·30 ml. 0·06 M-H_2O_2, then 0·20 ml. 10·5 M-H_2O_2 added from a second dangling tube giving 1·50 ml. 1·46 M-H_2O_2. Measurements made with Barcroft differential manometers at 20° in phosphate buffer (pH 5·85), concentration in each experiment 0·024 M.

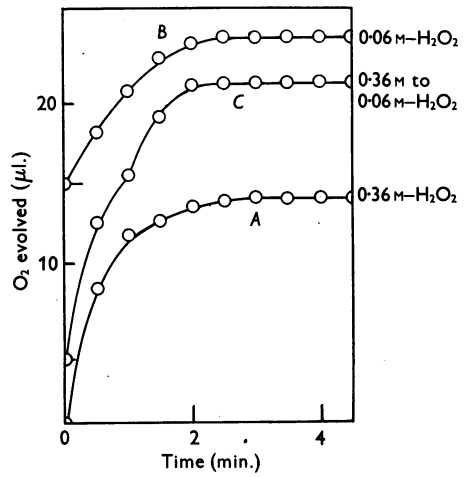

Fig. 7. O_2 evolved during the initial rapid reaction with 0·36 and 0·06 M-H_2O_2, calculated from the O_2-evolution curves in Fig. 3 by subtracting the O_2 evolved in the steady reaction. Curve *A* (from Fig. 3*A*): 0·36 M-H_2O_2, 0·25 ml. solution. Curve *B* (from Fig. 3*B*): 0·06 M-H_2O_2, 1·50 ml. solution. Curve *C* (from Fig. 3*D*): from 0 to 1 min. 0·25 ml. 0·36 M-H_2O_2, then 1·25 ml. dilute buffer solution added from a side arm giving 1·50 ml. 0·06 M-H_2O_2.

change in H_2O_2 concentration. The rates of O_2 evolution, measured over the period 2–4·5 min., from curves *B*, *A* and *C* are 10·9, 2·9 and 2·9 μl. O_2/min. respectively.

DISCUSSION

The experiments described above show that the rate of decomposition of H_2O_2 by catalase is directly proportional to the enzyme concentration and shows a complex variation with the H_2O_2 concentration. Below 0·06 M-H_2O_2 the rate is directly proportional, between 0·06 and 0·08 M-H_2O_2 there is a maximum in the rate, above 0·08 M-H_2O_2 further increase in the peroxide decreases the rate. These results, particularly the inhibition at high H_2O_2 concentration, are in no way determined by the purity of the enzyme, the presence of inhibitors in the

The experiment is one of the series in which dilute buffer solution was added during the reaction, the addition being made at the end of the first minute, i.e. during the period of the rapid reaction. The concentration change was from 0·36 to 0·06 M-H_2O_2. The effect of this dilution during the initial rapid reaction can best be seen by subtracting from all the experimental points in Fig. 3 the appropriate contribution of the steady rate. This is done in Fig. 7, where curves *A*, *B* and *C* give the O_2 evolution for the calculated rapid reaction for 0·36, 0·06 and 0·36 → 0·06 M-H_2O_2, from the experimental points in Fig. 3, curves *A*, *B* and *D*. The rapid reaction is over more quickly the higher the peroxide concentration. With 0·36 M-H_2O_2, 83 % is over in the first minute, with 0·06 M-H_2O_2, 65 %. Curve *C*, Fig. 7, shows that after dilution a greater quantity of O_2 is evolved in completing the initial rapid reaction than is evolved in the more concentrated solution. This suggests

that the transition to the steady rate cannot be due to any partial destruction of the enzyme. If it were, no increase in O_2 evolution would be observed on dilution. The explanation for this transition from α- to β-activity in the decomposition of H_2O_2 by catalase should be sought in the series of unit reactions involved in the peroxide decomposition. In this respect there is a similarity in the kinetics of the enzymatic decomposition and of the reaction catalyzed by ferrous iron (George, 1947), even though the iron in catalase is in the ferric state.

SUMMARY

1. The evolution of oxygen from hydrogen peroxide at pH 5·58 by liver catalase, erythrocyte catalase and a suspension of plasmolyzed red blood corpuscles has been measured manometrically, and by the 'boat technique' of Meldrum & Roughton (1934) using a pressure gauge for following rapid rates. There is an initial rapid evolution of O_2 followed by a slower steady evolution: a transition from α- to β-activity.

2. Identical kinetic relationships are obtained with the three specimens of catalase. The rate is directly proportional to the catalase concentration. The variation of the rate with peroxide concentration shows a maximum at about 0·07 M-H_2O_2, further increase in the H_2O_2 concentration decreasing the rate very markedly.

3. These kinetic relationships, particularly the inhibition at high H_2O_2 concentrations, are in no way determined by the purity of the enzyme, the presence of inhibitors in the peroxide or the nature and concentration of the buffer solution used.

4. The quantitative reversal of the inhibition shown by addition of dilute buffer solution or peroxide during the reaction shows that enzyme destruction plays no part in determining these kinetic relationships.

5. Dilution during the period of initial rapid activity shows a similar reversibility which suggests that the transition from α- to β-activity is not caused by the partial destruction of the enzyme.

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Activity of the Succinic Dehydrogenase-cytochrome System in Different Tissue Preparations

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The object of this paper is the study of the effect of different factors on the rate of aerobic oxidation of succinic acid by various tissue preparations. From the results obtained it is apparent that there are broadly speaking two types of factor: first, those which may react directly and specifically with one or more components of the succinic dehydrogenase-cytochrome system or of other catalytic mechanisms linked to this system; and secondly those which, by modifying the colloidal state of the enzyme preparations, may markedly increase or decrease their activity. The significance of the latter type of factor has not hitherto been fully recognized, with the result that the mode of action of a number of inhibitors and activators on the succinic system in tissue extracts has been misinterpreted, and the activators have erroneously been considered as essential links in the catalytic chain which brings about the aerobic oxidation of succinate. This study will enable us to examine critically the claims of